RENAL TUBULAR HANDLING AND DIURETIC EFFECT OF MERCURIAL DIURETICS

A Study in the Chicken

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som med vederbörligt tillstånd av Kansler för
Rikets Universitet för vinnande av medicine doktorsgrad
framlägges till offentlig granskning å Farmakologiska
Institutionens i Uppsala föreläsningssal
lördagen den 12 november 1960
kl. 9 f.m.

av

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Civiling., Med. kand.

R. UNIV.
BIBLIOTHEEK
LEIDEN

UPPSALA 1960

791/60

Almqvist & Wiksells
BOKTRYCKERI AKTIEBOLAG
UPPSALA 1960

Excerpt from Acta Societatis Medicorium Upsaliensis 65, 361 (1960)

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Renal Tubular Handling and Diuretic Effect of Mercurial Diuretics

A Study in the Chicken

by

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The present article comprises a summary of earlier investigations, which have been reported in the following papers:

I. CAMPBELL, D. E. S. and SJÖBERG, C.-I.: A device allowing a fraction collector to be operated in accordance with a preset, easily changeable collecting time program. *Scand. J. Clin. Lab. Invest. 12,* 362–366, 1960.

II. CAMPBELL, D. E. S.: Improved method for collecting and measuring ureteral urine flow in the chicken. *Acta pharmacol. et toxicol. 17,* 205–212, 1960.

III. CAMPBELL, D. E. S.: The excretion of mercaptomerin and its diuretic effect modified by bromcresol green and probenecid. *Acta pharmacol. et toxicol. 16,* 151–170, 1959.

IV. CAMPBELL, D. E. S.: Modification by bromcresol green or probenecid of urinary excretion of sodium, chloride and potassium due to mercaptomerin. *Acta pharmacol. et toxicol. 17,* 137–150, 1960.

V. CAMPBELL, D. E. S.: Modification by bromcresol green or probenecid of the excretion and diuretic effect of three mercurial diuretics: diurgin ®, chlormerodrin and mercumatilin. *Acta pharmacol. et toxicol. 17,* 213–232, 1960.

These articles will be referred to by the Roman numerals I–V in the following text. Preliminary results have been published as follows:

CAMPBELL, D.: Tubular secretion and diuretic action of mercurial diuretics. *Acta Physiol. Scand. 42,* suppl. 145, 27, 1957.

CAMPBELL, D.: Excretion and diuretic action of mercurial diuretics. *Experientia 13,* 327–328, 1957.

Introduction

Many substances containing mercury evoke diuresis. Only in a small group of chemically related organo-mercurials, however, is a high diuretic potency combined with a sufficiently low toxicity to make them clinically acceptable. From the chance discovery of the first of these substances the others were developed, mainly by the pharmaceutical industry, using chemical modification and screening. The oldest mercurial still in use is mersalyl, salyrgan ® (Bernheim, 1924; Brunn, 1924). Its important excretion characteristics were recognized by Møller (1930). When it is given intravenously to man, there is an extremely rapid appearance of large amounts of mercury in the urine. Møller obtained some evidence to show that this mercury still remained in the unchanged salyrgan molecule.

In view of this rapid excretion and the acidic character of mersalyl it was suggested by Brun, Hilden & Raaschou (1947) that mersalyl was excreted by tubular secretion. They inferred that consequently a particularly high concentration of mersalyl was obtained in the tubular cells, thus causing diuresis by reduced reabsorption of water and ions.

Weiner *et al.* (1956), using low doses of mersalyl giving no diuretic response, showed that mersalyl was excreted by tubular secretion. They employed the method developed by Sperber (1946, 1948) for the study of tubular secretion in chickens. This method makes use of the renal portal circulation present in birds, which allows a study of tubular secretion without determination of any plasma concentrations. When the paper of Weiner *et al.* became available (Jan. 1957), the present investigation, also using the Sperber method, had already been started.

By using larger doses of mercurials, which also evoked a diuretic response, tubular secretion could be studied in relation to the diuretic response. It was soon found that inhibition of the tubular secretion system also modified the diuretic response.

Methods

The technique used was based on that developed by Sperber (1946, 1948; Lindahl & Sperber, 1956). The renal portal circulation which

exists in the bird kidney, causes substances introduced unilaterally into one leg vein to be partly taken up from the blood by the tubules in the kidney of the injected side before entering the general circulation. If the uptake is rapid enough, an excess excretion on the injected side can be obtained. In previous studies using this preparation the total volume of urine found was of secondary importance and not recorded.

In the present study it was considered of interest, however, to measure both the amount of mercurial and the amount of urine from each kidney. It was therefore necessary to modify the urine collection arrangement to enable a quantitative measurement of the flow of urine to be made. Paper II contains a description of the details of the arrangement as it was developed.

Urine was collected by two plastic funnels which were sewn into the cloaca of the chicken and kept free from occluding clumps of uric acid by two streams of rinsing water, exactly as described by Lindahl & Sperber (1956). To be able to measure the voided amount of urine, however, it was necessary exactly to determine the amount of rinsing water added. This was achieved by keeping the rate of the two rinsing flows and the length of the collecting periods extremely constant. The amount of urine + rinsing water gathered during each collection period was accurately measured by weighing, and a calculated amount of rinsing water was subtracted.

To obtain the exact timing of the collection periods a fraction collector was used. A conventional fraction collector changes the collecting tubes after constant intervals of time, but in the present investigation it was essential to be able to vary the collecting times in a simple way during the experiment, and yet maintain a high standard of precision in timing. A commercial fraction collector was modified and a new timer developed (I) to allow any of 4 different collection times, changeable at very short notice, to be used for each individual collection period. The arrangement was very reliable and saved a lot of handling time and the standard of timing precision was high enough to avoid any measurable error in the determination of diuresis. Some practical rearrangements of the chicken support and precautions against the risk of fecal contamination also became necessary (II).

With the apparatus used (I and II) it was possible to collect and measure the flow of urine continuously during experiments lasting a whole day; the urine was collected quantitatively. The accuracy in the measurement of the rate of flow was related to many factors and was not immediately determinable. Some analyses are presented in (II) to elucidate the degree of accuracy to be expected. A series of experiments carried out under the same conditions as for the chicken experiments,

in which the rinsing flow alone was collected showed that the total collecting variability (standard deviation) for any one 20-minute sample was of the order of ± 0.0023 ml/min. This figure should be related to urine flowing from one ureter of an average hen at a basal rate of 0.015 ml/min.

To reduce the effect of variability in the determination of the rate of basal flow in the mercurial diuretic experiments (III, IV, V) the basal flow of urine was determined as the average of 5–12 20-minute collecting periods, taken both before any injection and after the mercurial diuresis had disappeared. At the start of the average experiment the mean flow of urine was obtained during 4.2 20-minute periods of stable flow; the values from those periods which gave markedly higher flows immediately after application of the funnels were discarded. At the end of 80% of the experiments the diuresis had returned to the level of the original basal flow, giving on the average 2.2 periods of final basal flow.

In the remaining 20% of the experiments in which the flow did not return to the basal level, basal flow was determined only from the periods before injection. In these experiments part of the diuretic effect was obviously never recorded, but extrapolation seems to show that this formed only a negligible fraction.

After the injection of mercurials urine was collected at 10-minute intervals, usually with little error, as a large increase in the flow of urine was often obtained. By adding together the volumes of urine obtained from the start of the injection of mercurial until the end of active diuresis, and subtracting a calculated contribution from a mean basal urine flow during this time, a measure in ml of the diuretic effect of the mercurial was obtained.

In the specimens containing urine and rinsing water collected during the mercurial diuretic experiments (III, V) the concentration of mercury was determined by a slight modification (III) of an as yet unpublished method of Vesterberg & Sjöholm.

Results

Mercurial Diuretics in the Chicken

No previous recordings of mercurial diuresis in chickens seem to have been made. Consequently the first experiments involving the injection of mercurials into chickens took the form of a comparative survey. Eight different mercurial diuretics in clinical use were injected into the leg vein at different rates and at different dose levels. Excretion of water and mercury was recorded.

Some of these results were given in the preliminary reports. Injection during a period of 1–10 min into one leg vein gave an excess excretion of mercury from the injected side in six of the eight mercurials. The highest excess was obtained with mercaptomerin, followed by diurgin ®, with the next highest. A slight excess was obtained with chlormerodrin and no excess with mercumatilin. The rate of mercury excretion also varied: mercaptomerin showed the most rapid excretion, followed by diurgin ®, chlormerodrin and mercumatilin.

By using proper doses, a diuretic response of water was obtained with all the mercurials. Injection of mercaptomerin into a leg vein during a period of 1–10 min gave a strictly unilateral diuresis, and when diurgin ® and chlormerodrin were used, diuresis was obtained predominantly on the injected side. With the exception of chlormerodrin, which seemed to have a higher potency than the others, the mercurials were roughly equipotent. The diuresis of water was accompanied by a simultaneous diuresis of sodium and chloride.

These mercurials thus seemed to evoke diuresis and excretion of mercury in the hen in the same way as in other species, such as dog and man.

Among the mercurials, mercaptomerin was chosen for the most intensive study because of its high degree of tubular secretion and rapid excretion.

Inhibition of Mercaptomerin Secretion and Diuretic Response of Water (III)

To inhibit the tubular secretion of mercaptomerin two different compounds were used: bromcresol green and probenecid.

Bromcresol green, a sulfonated dye, was shown by Sperber (1954) to be a potent competitive inhibitor of the secretion of many other sulfonated dyes transported by the *p*-aminohippurate transport system. It is in itself secreted by both the liver and the kidneys, and for this reason leaves the body quite rapidly. In contrast, the other inhibitor used, probenecid which also affects the hippurate transport system is very slowly excreted (Beyer *et al.*, 1951). It has recently been shown that this substance is rapidly secreted and subsequently very efficiently reabsorbed by the tubules (Weiner *et al.*, 1960).

Two experiments were carried out on each hen, namely one inhibition and one control experiment; in both experiments the same dose of mercaptomerin was given in the same leg vein. In the inhibition experiment one of the inhibitors was injected systemically to give a full inhibiting effect when the mercurial was introduced. In the control experiment there was no inhibitor present to affect the mercaptomerin

when it was introduced, but a control injection of the inhibitor was made either later or much earlier.

In the control group of 5 chickens in which there was no inhibitor present a single leg vein injection of mercaptomerin gave without exception an excess excretion of mercury on the injected side. Within 80 min, when the excess excretion had ceased and a total of 86% of the dose had left the body, a mean excess of 24% of the dose was obtained. In every experiment diuresis of water on the injected side was larger than on the non-injected side.

In every experiment on 10 chickens symmetrical pre-injection of bromcresol green caused a strong reduction of the excess excretion of mercury on the injected side. The excess diuresis of water was noticeably reduced in 9 experiments. Thus it seemed that bromcresol green blocked in a striking way both tubular transport and the effect of mercaptomerin injected into the renal portal blood of one side.

Control experiments using bromcresol green given immediately after the mercurial showed normal unilaterality of mercury excretion and unilaterality of diuresis which was at least normal. Consequently bromcresol green did not interfere with the transport within the cell or with the diuretic response to mercurials as such, but with the rapid uptake of the diuretic by the tubules.

Inhibition by probenecid gave the same results: total suppression of the normal excess diuresis of water and of excess excretion of mercury. A control injection of probenecid after the mercaptomerin led to normal Hg-excess excretion and possibly to an increase in excess diuresis.

It can be concluded from the experiments that mercaptomerin is taken up by the tubules by an active process which is inhibitable by inhibitors of the hippurate transport system. This applies both to that portion of the diuretic which is rapidly excreted and to that portion which is diuretically active.

Inhibition of Mercaptomerin Diuresis of Sodium and Chloride Ions (IV)

Mercaptomerin diuresis was recorded in (III) only as diuresis of water. In order to trace the main ions in the mercurial diuresis all the urine specimens obtained in (III) were analysed for their contents of Na and Cl ions. Some of the specimens were also analysed for K ions.

The controls which received unilateral leg-vein injection of mercaptomerin alone produced a unilateral diuresis of Na and Cl ions at the same time as the water diuresis but of a much more pronounced nature.

Additional experiments to investigate the unilateral diuresis showed that it was efficiently blocked by dimercaprol (BAL), which is known to block mercurial diuresis in other species. The diuresis seemed not to have been caused by a unilateral increase in the glomerular filtration rate. Systemic injection of mercaptomerin produced diuresis of the same order as was produced by unilateral injection.

The inhibition experiments showed the same main results as had been obtained for water. If mercaptomerin was unilaterally injected when either inhibitor had taken full effect, the normal excess diuresis of Na and Cl was completely abolished. If an inhibitor was injected shortly after the mercaptomerin, normal excess diuresis resulted. The same conclusion as in (III) is made, i.e., that the inhibitors prevent the rapid uptake of mercaptomerin from the blood by the tubules.

The inhibitors also evoked bilateral effects which were clearly visible in the Hg excretion and the diuresis from both sides added together. The Hg excretion results tally very closely with those for diuresis of Na and Cl ions. Diuresis of water is complicated by the injection of the inhibitor solution which in itself causes a symmetrical diuresis. If this contribution is taken into account the inhibition effects given below seem to be equally applicable to the diuresis of water.

Inhibition by bromcresol green caused a bilateral Hg excretion and diuresis of Na and Cl ions of essentially normal size but noticeably delayed in appearance. In contrast, probenecid caused a sustained retardation of the bilateral Hg excretion and a totally suppressed diuretic effect.

These results can be explained by an inhibition of the tubular uptake of mercaptomerin symmetrically in both kidneys. With bromcresol green the inhibition is only temporary and within a short time normal conditions have returned. With probenecid a sustained inhibition is obtained, due to the prolonged action of probenecid.

Inhibition of Hg Excretion and Diuresis Using Three Other Mercurials (V)

It was of interest to extend the results obtained with mercaptomerin (III, IV) to other mercurials. Among those available three were chosen. Diurgin ® is closely related to mercaptomerin in one respect; one valency of mercury is bound to a thiol group in both. With respect to the group bound by the other Hg valency they are, however, quite different. While diurgin ® contains an acyclic residue, mercaptomerin contains an isocyclic one. Chlormerodrin is a derivative of urea and has a higher diuretic potency than any other mercurial (Handley *et al.*, 1951; Moyer *et al.*, 1952). Mercumatilin has in a sense one of the most heterologous structures of the clinically used organo-mercurials, since

it lacks the –CO–NH– link present in most of the others. Some experiments using inorganic mercury in the form of $HgCl_2$ were also included for comparison. The apparatus and methods used previously (III) were also employed here. Only diuretic response of water was recorded.

Rapid unilateral injection into a leg vein of diurgin ® and chlormerodrin gave an excess excretion of Hg and an excess water diuresis on the injected side in every control experiment. The unilaterality seemed, however, to be less than with mercaptomerin. Rapid leg vein injection of mercumatilin caused only slight unilaterality. With slow infusion of mercumatilin into a leg vein during a period of 30 min it was, however, possible to obtain in every experiment a considerable excess excretion of Hg and an excess diuresis of water. Thus all these mercurials are to some extent taken up from the blood by tubules on the injected side during their first passage.

Inhibition experiments using bromcresol green caused throughout a reduction of the excess excretion of Hg for all three mercurials. A reduction in excess diuresis of water was also obtained for all the mercurials, although the significance of the results on chlormerodrin was small. In spite of inhibition mercumatilin, in contrast to the other mercurials, showed a residue of unilaterality as witnessed by both a small excess excretion of Hg and a small excess diuresis of water.

Experiments in which bromcresol green was injected after the mercurials showed no effect on the normal unilaterality of Hg excretion and water diuresis.

Inhibition by probenecid gave a reduction of unilaterality for all the mercurials in every experiment. Mercumatilin again, however, gave a small residue of excess excretion of Hg and an indication of some excess diuresis of water.

From the reduced unilaterality it is thus evident that the inhibitors are able to inhibit the tubular uptake of the three different mercurials from the blood on their first passage through the kidney.

The tubular uptake was inhibited so deeply and for such a long time that the effects became clearly visible for all three mercurials also in the bilateral excretion and diuresis. Inhibition by bromcresol green gave a temporal retardation of Hg excretion and a temporal retardation of water diuresis. Inhibition by probenecid caused a sustained reduction of Hg excretion and a definite reduction of water diuresis from both sides which was not compensated. As these effects are obtainable irrespective of the unilaterality of urine collection, they should also have been visible after systemic injection of the mercurials in the hen. It seems probable that these effects are also obtainable in other species.

In the experiments in which unilateral injection of inorganic mercury in the form of $HgCl_2$ was used, urinary excretion of mercury was

so slow that it could not be followed. A more variable diuretic response was found, but as a rule an excess diuresis on the injected side was obtainable. In spite of pre-injection of probenecid, unilateral injection of $HgCl_2$ evoked, as a rule, an excess diuresis. It is to be concluded that mercurial diuresis may also be initiated by mechanisms which are not inhibitable by potent inhibitors of the hippurate transport system and that probenecid does not interfere with mercury-induced diuresis as such.

Tubular Secretion as a Factor in the Action of Mercurial Diuretics

The extensive literature, which has been devoted to mercurial diuretics throws a good deal of light on various aspects of their mechanism of action. This literature has frequently been reviewed, for instance, by Engel & Epstein (1931), Pitts & Sartorius (1950), Vogl (1953), N. Y. Acad. Sci. Conference (1957) and (1958), Pitts (1959), and Kruhøffer (1960). As these papers include recent details of the topic, only some of the more important characteristics will be briefly mentioned here.

It is generally acknowledged that mercurial diuresis is the result of an inhibition of the tubular reabsorption of water, sodium and chloride. In turn, this inhibition is believed to be caused by the mercurial inhibiting some enzyme essential to the active transport of sodium or chloride, or both. Conclusive evidence of specific enzyme inhibition seems, however, still to be lacking (Bickers *et al.*, 1960).

A large diuresis can be obtained by the use of simple inorganic mercurials. Thus $HgCl_2$ can give rise to a concentration of an active mercurial complex in the tubular cell which will cause a proper inhibition of reabsorption and a large diuresis (Kessler *et al.*, 1957), (V). The main effect of those mercurials in general clinical use is to give not a larger but a much more reliable diuresis and far fewer toxic effects. It is considered that a higher degree of enzyme inhibition is the cause of the toxic effects elicited by the mercurials.

As suggested by Lehman *et al.* (1951) and later stressed by Hughes (1957), for physico-chemical reasons the carbon–mercury bond in a mercurial is stable under physiological circumstances, while the non-carbon–mercury bond is unstable, reacting with the thiol groups in the body to form a mercury–sulfur bond. The extremely large association constant for this reaction means that the mercurial will be rapidly and almost completely bound to thiols.

This hypothesis, difficult to prove conclusively *in vivo,* is consistent with a number of observations. The mercurials are very strongly plasma-bound. They are excreted almost exclusively as a complex of the original organo-mercurial with thiols (Weiner & Müller, 1955; Müller & Weiner, 1956; Leuschner, 1957; Handley *et al.,* 1956). Mercurials in order of increasing bond-stability Cl–Hg–Cl, R–CH_2–Hg–OH (R = organic residue), R–CH_2–Hg–N (–N in theophylline), R–CH_2–Hg–S (–S in thiol group) show in the same order decreasing ability to cause local toxic reactions (shown for the Hg–N and Hg–S bond by Lehman *et al.,* 1949) and an increasing rate of local absorption (shown for the Hg–O, Hg–N and Hg–S bond by DeGraff *et al.,* 1938 and Lehman *et al.,* 1950).

In their distribution all mercurials show some degree of concentration to the renal cortex (Hughes, 1957; Kessler *et al.,* 1957); the degree of this concentration is little related to the diuretic potency (Kessler *et al.,* 1957).

The relation between the diuretic effect and the renal excretion of the compound itself was first discussed for mersalyl by Brun *et al.* (1947). For various reasons they thought it probable that the excretion occurred by tubular secretion and consequently inferred that a high concentration of the mercurial was obtained in those tubular cells in which the reabsorption of water and ions takes place.

According to more recent concepts of the secretion process this can be divided into at least two stages: an uptake step and an excretion step (Shannon, 1939; Josephson, 1950; Josephson & Kallas, 1953; Puck *et al.,* 1952).

It now seems to be an established fact that rapid transcellular secretion does not necessarily correspond to a high intracellular concentration. The secretion of, e.g., phenol red by the isolated renal tubules of the flounder (Puck *et al.,* 1952) may proceed rapidly without observable intracellular concentration, thus showing that the excretion proceeds fast enough at low intracellular concentrations to keep pace with the uptake.

If Brun *et al.* had written their paper to-day, they would probably have talked in terms of an uptake step and not of secretion as such.

Several discoveries have since been made which link tubular secretion of the mercurials more closely with diuretic effects. Weiner *et al.* (1956), Campbell (1957) and (III) and Kessler *et al.* (1958) have all produced evidence to show that tubular secretion of different mercurials does take place.

Kessler *et al.* (1957) found in experiments with the dog that among 3 mercurials in clinical use and 9 other mercurials only those which were rapidly excreted in the urine also gave a diuretic response. There

was, however, one remarkable exception: *p*-chloromercuribenzoate, which gave no diuresis although it was rapidly excreted.

From their paper and from Pitts' book (1959) it would seem that they considered that the secretion of mercurial diuretics took place by a special secretion system different from that for *p*-aminohippurate. However, it was only the excretion of *p*-chloromercuribenzoate that was studied after inhibition of the hippurate transport system by probenecid, and it was still found to be excreted at a high rate. Kessler *et al.* were thus unable to separate the transport system of *p*-chloromercuribenzoate from that shared by the other mercurials and in their generalisations they discarded the theory that tubular secretion was essential for diuresis. A statement by Kessler *et al.* (1958) that probenecid does not influence the secretion of chlormerodrin in the dog was based on the experiments with *p*-chloromercuribenzoate alone (Kessler, personal communication).

From the inhibition experiments presented here (III, IV, V) it would appear probable that all of four different mercurials in clinical use were secreted by the *p*-aminohippurate system. Where the uptake step was inhibited, the diuresis was also inhibited. Thus, a normal course of the process which seems to be the first step in the tubular secretion of these mercurials would seem to be equally essential for their diuretic activity.

Once the common secretion system of the mercurial diuretics has been localized to the hippurate system, the apparent irregularity of *p*-chloromercuribenzoate is explained, since Kessler *et al.* (1957) showed that the rapid excretion of this substance is not inhibitable by probenecid. Consequently it is an exception in that it does not share the common secretory mechanism.

It is thus evident that for at least some of the mercurials in clinical use an active tubular uptake is essential for diuretic effect after ordinary doses. However, there are presumably other means by which mercury can reach the tubular cells to cause a diuretic effect. In experiments on the hen diuresis can be obtained by $HgCl_2$, although no rapid excretion of Hg occurs, as it does in experiments on the dog. A rapid, active tubular uptake of $HgCl_2$ by the *p*-aminohippurate system seems rather unlikely; and pre-treatment with probenecid did not consistently inhibit the diuresis (V). It is suggested that this mercury enters the tubular cells by simple diffusion, after which it evokes diuresis there. For one mercurial, mercumatilin, the effect of the inhibitors was incomplete, which also suggests tubular action without active uptake at least by the hippurate system.

In an attempt to relate the structure of mercury compounds to their diuretic effect, they have been classed either as diuretics or as non-

diuretics. Attempts have been made to correlate this differentiation either with acid lability (Mudge & Weiner, 1958) or with a special key configuration for diuretic activity (Kessler *et al.*, 1957). According to our results, it is also necessary to take into account the structural configuration consistent with active tubular uptake.

Acknowledgement

This work, inspired by Professor Ernst Bárány, M.D., Head of the Department of Pharmacology at the University of Uppsala, was made possible by all the facilities he placed at my disposal. I wish to express my deep gratitude to him for his continuous personal interest, illuminating criticisms and fund of good advice.

My sincere thanks are due to Professor Ivar Sperber, Ph.D., at the Institute of Animal Physiology, Royal College of Agriculture, Ultuna, who introduced me to his special field of renal physiology and taught me his remarkable technique in using the kidney of the chicken.

I am indebted to Assistant Professor R. Vesterberg at the National Institute of Public Health, Stockholm, for placing at my disposal his method of mercury determination in biological material.

The staff of the Department of Pharmacology were most helpful throughout the course of this investigation. In particular I wish to thank Miss Ingalill Dalhagen, Miss Yvonne Larsén and Mrs. Inger Snickare for their skilful collection of the experimental material, Mr. Manne Fredriksson and Mr. Folke Högberg for constructing and maintaining the experimental arrangement and Mrs. Ann-Mari Kjellberg for repeatedly typing the manuscript. I am grateful to Mr. Claes-Iwar Sjöberg for designing and building the electrical equipment, his efforts in "trouble-shooting" and his collaboration in Paper I. Mr. Michael Knight kindly revised the English text.

I am indebted to Astra Ltd., Södertälje, and Pharmacia Ltd., Uppsala, for generous supplies of mercurials.

This work was supported by grants from the Medical Faculty of the University of Uppsala, the Svenska Sällskapet för Medicinsk forskning and Svenska Nationalföreningen mot Tuberkulos.

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